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Carotene Loss in Stored Leaf Meals and Extracts

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Carotene retention in vegetable leaf meals depends primarily on the storage temperature. Of the three temperatures used in these tests (5°, 24°, and 37.5° C.), the greatest carotene stability was at 5° and the least at 37.5°. Retention varied with the species of leaf, and a high oxalic acid content of the leaf seemed to be favorable. Carotene concentrates from leaf meals dissolved in oils are more stable than crystalline carotene in the same oils, possibly owing to the tocopherol in the concentrate. Levels of carotene up to 1000 micrograms per gram are more stable than the 5000- and 15,000-microgram levels. Oil solutions of carotene, of whatever source and at any concentration, are in most cases more stable at 5° C. than at higher temperatures. Addition of *d*-isoascorbyl palmitate and soybean lecithin to the carotene solution is ineffective in reducing loss of carotene.

CERTAIN dry vegetable leaf meals can be prepared which contain large amounts of carotene (*6*). These meals can be used as poultry feed supplements (*15*) or as starting material for carotene concentrates (*18*). Since both the leaf meals and oil solutions of the carotene concentrate may often be held in storage for considerable periods, the carotene losses of these products under various storage conditions were investigated.

Leaves of beet, carrot, broccoli, lima bean, rhubarb, spinach, and sweet corn were dried to a residual moisture content of 5% and ground 30 to 40 mesh in a Wiley mill. Prior to drying, portions of each material were blanched with steam at 100° C. or hot water at 90° to 100° for 5 minutes. The ground leaf meals were stored in 4-ounce screw-capped bottles; some were also stored in cloth bags. The meals were kept in the dark at 5°, 24°, and 37.5° C.

A carotene concentrate was prepared from a mixture of a number of leaf meals. The mixture was extracted with Skellysolve B; the extract was saponified to remove chlorophyll, and finally purified with hydrated lime according to the procedure of Wall, Kelley, and Willaman (*18*). Removal of the solvent gave the carotene concentrate used for all the "concentrate" experiments.

Aliquots of the concentrate, dissolved in Skellysolve B, were mixed with crude soybean and peanut oils; refined cottonseed, corn, soybean and peanut oils; lard, Crisco (a commercial shortening made from hydrogenated vegetable oil), and mineral oil. Aliquots were taken to give 100, 1000, 5000, and in some cases 15,000 micrograms of carotene per gram of oil. The solvent was removed on a warm water bath under nitrogen and vacuum. The oil solutions were stored in screw-capped vials in the dark at 5°, 24°, and 37.5° C. In the concentration range 100 to 5000 micrograms, 5 grams of oil solution were stored in 60 × 17 mm. vials. At the 15,000-microgram range concentration, 2 grams of oil solution were stored in 45 × 15 mm. vials. Samples were analyzed for carotene over a period of 6 months, using single determinations. Similar studies were made with oil solutions of crystalline carotene prepared in the same manner as the carotene concentrate solutions.

The source of crystalline carotene was S.M.A. Corporation's 90% beta, 10% alpha carotene. The crystalline carotene solutions were prepared from carotene stored in vacuum-sealed vials and were used at once. In all cases carotene was determined by the method of Wall and Kelley (*17*). Since some isomerization of carotene conceivably occurred during storage, and since some of these products may not be separated from beta carotene by the method used, the points in the curves probably represent maximum values in terms of physiological activity.

CAROTENE RETENTION IN DRY LEAF MEALS

Data on stored leaf meals are shown in Figure 1. Only the results of experiments conducted in screw-capped bottles are presented; the trend of the bag experiments was similar. At least three factors are involved in carotene retention in dry leaf meals—time, temperature, and nature of leaf. Other factors, such as light and moisture content, were not involved in the present experiments. As shown in Figure 1 and by other data not presented here, blanching in the manner described can be eliminated as a factor in the carotene retention of these meals during storage.

The storage temperature markedly affected the rate of carotene loss; time was a secondary factor. The rate of decomposition was slowest at 5° C., increased at 24°, and was even more rapid at 37.5°. The rate and extent of carotene loss varied considerably among the leaf meals. In respect to carotene retention, the leaf meals may be divided into three groups: (*a*) high—beet top, spinach, and rhubarb; (*b*) medium—broccoli and sweet corn; (*c*) low—carrot and lima bean. After 6-month storage, group *a* retained 70% carotene at 5° C. and 40% at 24°; group *b*, 60 and 25%, respectively; and group *c*, 30 and 15%, respectively. There may be some significance in the fact that all three leaf meals showing maximum carotene retention were very high in oxalic acid, whereas the leaf meals in groups *b* and *c* contained little oxalic acid.

CAROTENE RETENTION IN OIL SOLUTION

The effects of time, temperature, concentration and source of carotene, and kind of oil were studied in determining carotene retention in oil solution. In addition, some studies were made of the effect of adding to the oil solutions an antioxidant consisting of a mixture of 0.06% *d*-isoascorbyl palmitate and 0.03% soybean lecithin. These factors will be discussed individually, but the stability of carotene depends on a combination of all of them.

The effect of length of storage on carotene stability is shown in Figures 2, 3, and 4. Under most conditions the rate of carotene loss increased as the storage period became longer. However, as will be shown later, under certain circumstances, such as low temperature and low carotene concentration, the time factor became negligible.

Figure 2 shows the effect of temperature on the stability of carotene concentrate in oils. As the temperature was increased from 5° through 37.5° C., the destruction of carotene in most cases became more rapid. Data for crystalline carotene in oil, not presented in Figure 2, indicate that temperature increments exerted even more effect on the rate and degree of carotene destruction. As shown in Figure 2 and by other data not presented, carotene in the range 100 to 1000 micrograms stored at

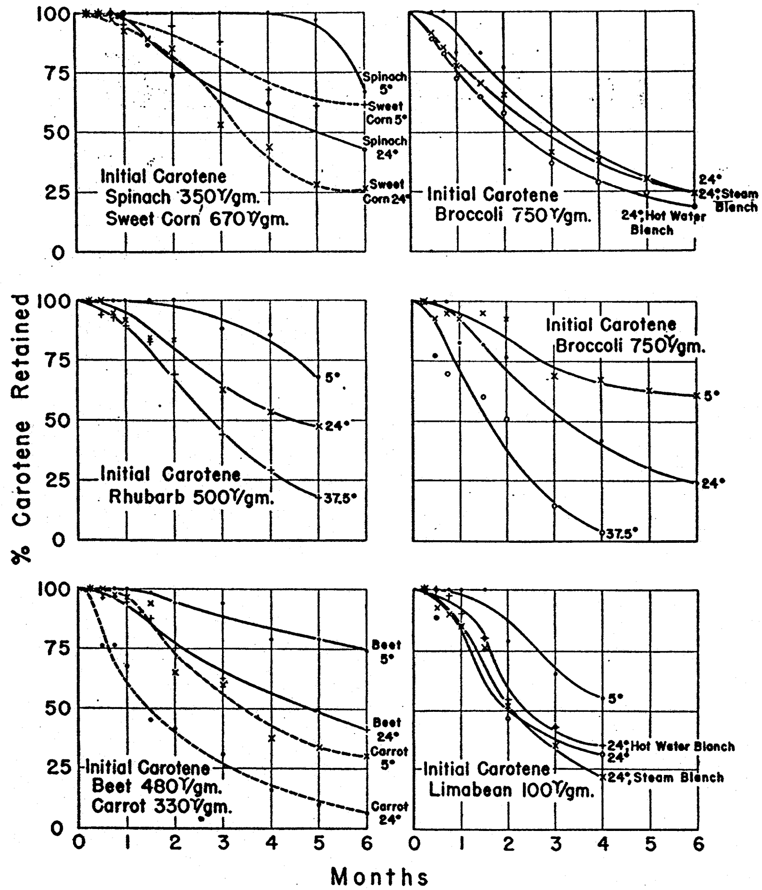

Figure 1. Effect of Storage Temperatures and Blanching on Carotene Retention in Dry Vegetable Leaf Meals

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5° C. was very stable; with few exceptions, 90 to 100% of the original carotene was retained at the end of 6 months.

Figure 3 illustrates the effect of carotene concentration on its stability in oils. Only the data for the carotene concentrate in oil at 24° C. are presented; results at other temperatures and with crystalline carotene showed similar trends. The low carotene samples, 100 to 1000 micrograms, were almost invariably more stable than the 5000–15,000 microgram series. The rate of carotene loss was slower at 5° C. and faster at 37.5°, but the general effect of concentration was the same at these temperatures. However, there was no regular concentration effect within the groups themselves. These irregularities may have been due to the nature of the various oils used, for the order of stability within the groups varied from oil to oil.

The data in Figure 4 show the effect of source of carotene on its stability in oils. The carotene concentrate was much more stable than the corresponding crystalline preparation. This was especially true with high carotene concentrations, such as 5000 micrograms per gram of oil. With lower concentrations this trend was not so regular, possibly because many of the oils may have contained sufficient natural antioxidants to give the crystalline carotene some protection. The superiority of the vegetable carotene concentrate is shown markedly in Figure 4, especially with mineral oil. The mineral oil used in this experiment contained no natural antioxidants. Crystalline carotene was completely decomposed at all temperatures and concentrations in one month, whereas the carotene concentrate was quite stable. These results indicate that the vegetable carotene concentrate contained natural antioxidants, the nature of which will be discussed later.

Figure 5 shows the percentage of carotene retained by the oil solutions of carotene concentrate and of crystalline carotene after 4-month storage at various temperatures. In most cases the nature of the oil was not significant. The carotene concentrate was stable in oils other than lard and even in mineral oil. Crystalline carotene, however, was very unstable in lard and mineral oil, and less stable than the concentrate in other oils.

In one series 0.06% *d*-isoascorbyl palmitate and 0.03% soybean lecithin were added to oil solutions of carotene. At 5°, 24° and 87.5° C. these additives had no significant effect on the stability of carotene.

DISCUSSION

The problem of carotene retention in vegetable leaf meals is one of preventing oxidation of a highly unsaturated and therefore relatively unstable compound. A number of workers (*4, 10, 13, 19*

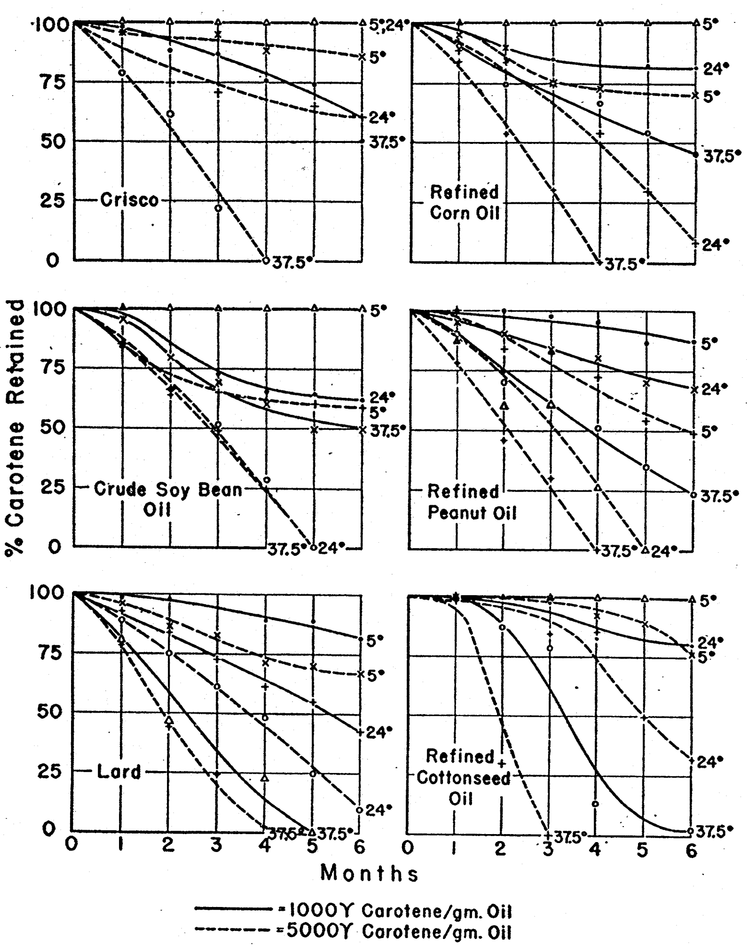

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Figure 2. Effect of Temperature on Stability of Carotene Concentrate in Oils

Figure 3. Effect of Carotene Concentration on Retention in Oils at 24° C.

Carotene concentrations in micrograms per gram of oil

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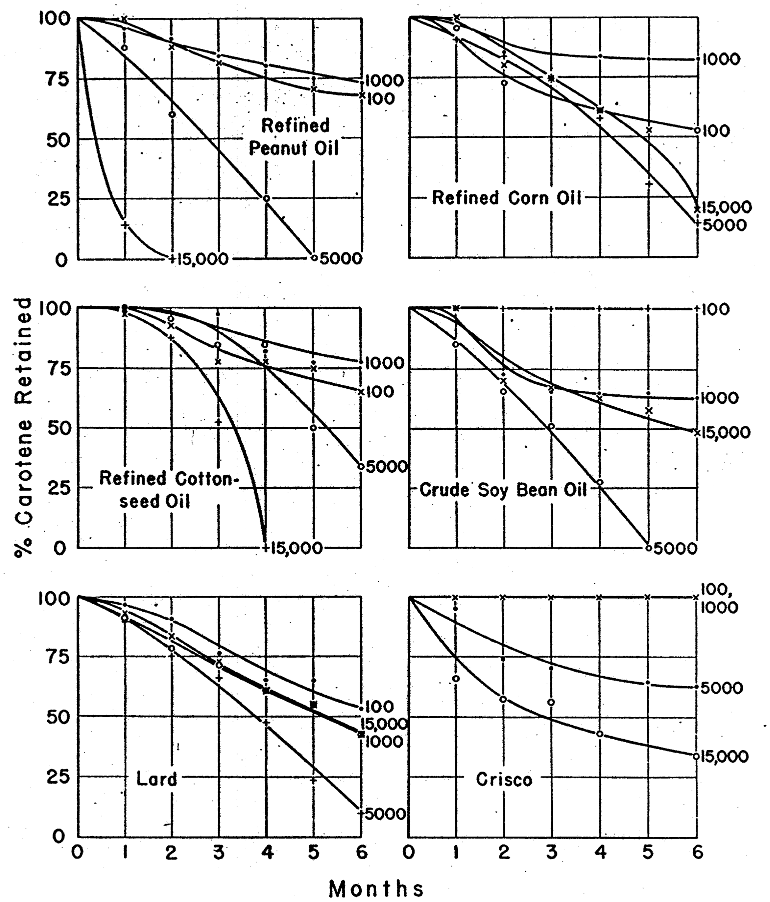

testing the stability of carotene in alfalfa, have found that losses increase with increased storage temperature. Dutton, Bailey, and Kohake (*3*), working with dehydrated spinach, demonstrated that carotene losses could be decreased by storage in an inert atmosphere such as carbon dioxide or nitrogen, and Taylor and Russell (*14*) reported no carotene losses in alfalfa stored in vacuum for 20 months at 0° to 5° C. Our findings that carotene losses in vegetable leaf meals increase with increased storage time and temperature are therefore similar to results obtained with alfalfa.

The fact that the vegetable leaf meals which had maximum carotene stability were very high in oxalic acid is of considerable interest. Mattill (*8*) emphasizes the fact that oxalic acid can act not only synergistically in the presence of phenolic antioxidants but as a stabilizer against rancidity when added alone to vegetable fats. It is conceivable that the increased carotene retention of these leaf meals is due, at least partially, to their high oxalic acid content.

The storage of carotene in oils presents problems similar to those encountered with leaf meals. A number of workers (*1, 7, 16*) have found that carotene losses of such oil solutions increase with lengthened storage periods and increased temperatures. The effect of wide variations in carotene concentration on carotene stability has not been extensively studied. Bickoff and Williams (*2*) showed that in pelleted mixtures of mineral oil and rice bran, carotene losses increased rapidly as the concentration rose from 200 to 1000 micrograms per gram. Morgal, Byers, and Miller (*9*) presented evidence that an alfalfa carotene concentrate is more stable in various media than is crystalline carotene.

Our conclusions therefore agree with those of other workers as to the effects of time and temperature on carotene losses in oil solution. In oils we found no significant differences from the results of Bickoff and Williams in the concentration range reported by them, but our carotene losses increased greatly at a concentration of 5000 micrograms per gram. Our comparison of the relative stability of carotene from a vegetable leaf concentrate and crystalline carotene shows that the concentrate was much more stable. This is similar to the results of Morgal *et al.* with an alfalfa concentrate. Unpublished data from this labora-

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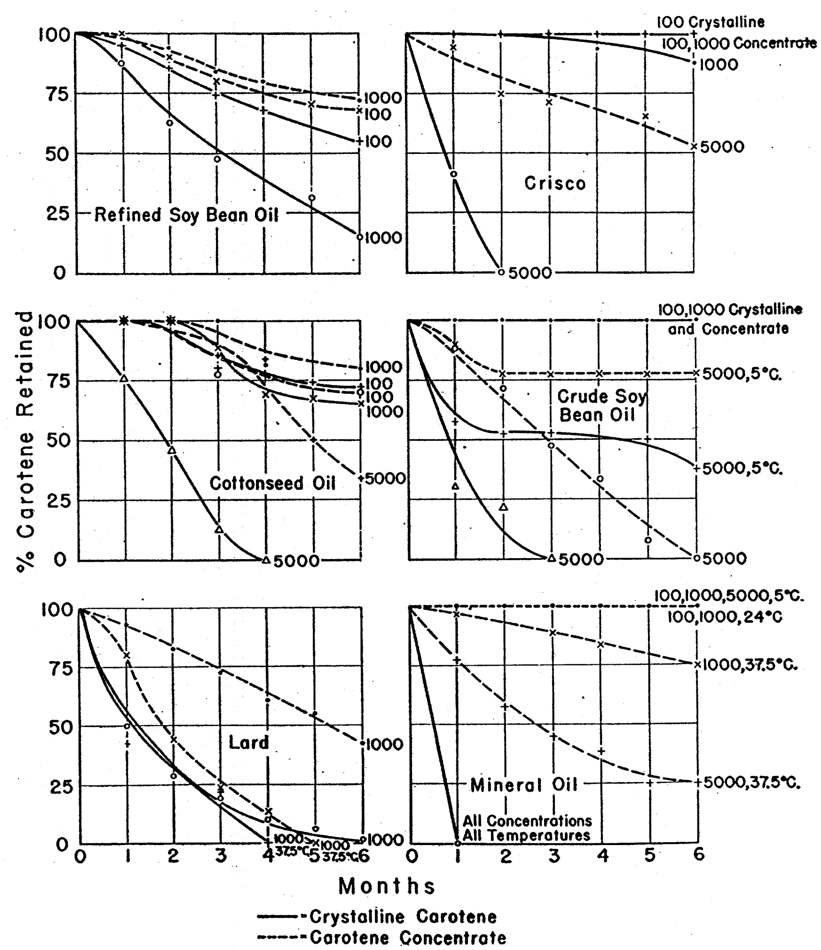

Figure 4. Effect of Source of Carotene on Retention in Oils

Carotene concentrations in micrograms per gram of oil; storage temperature, 24° C. unless stated otherwise

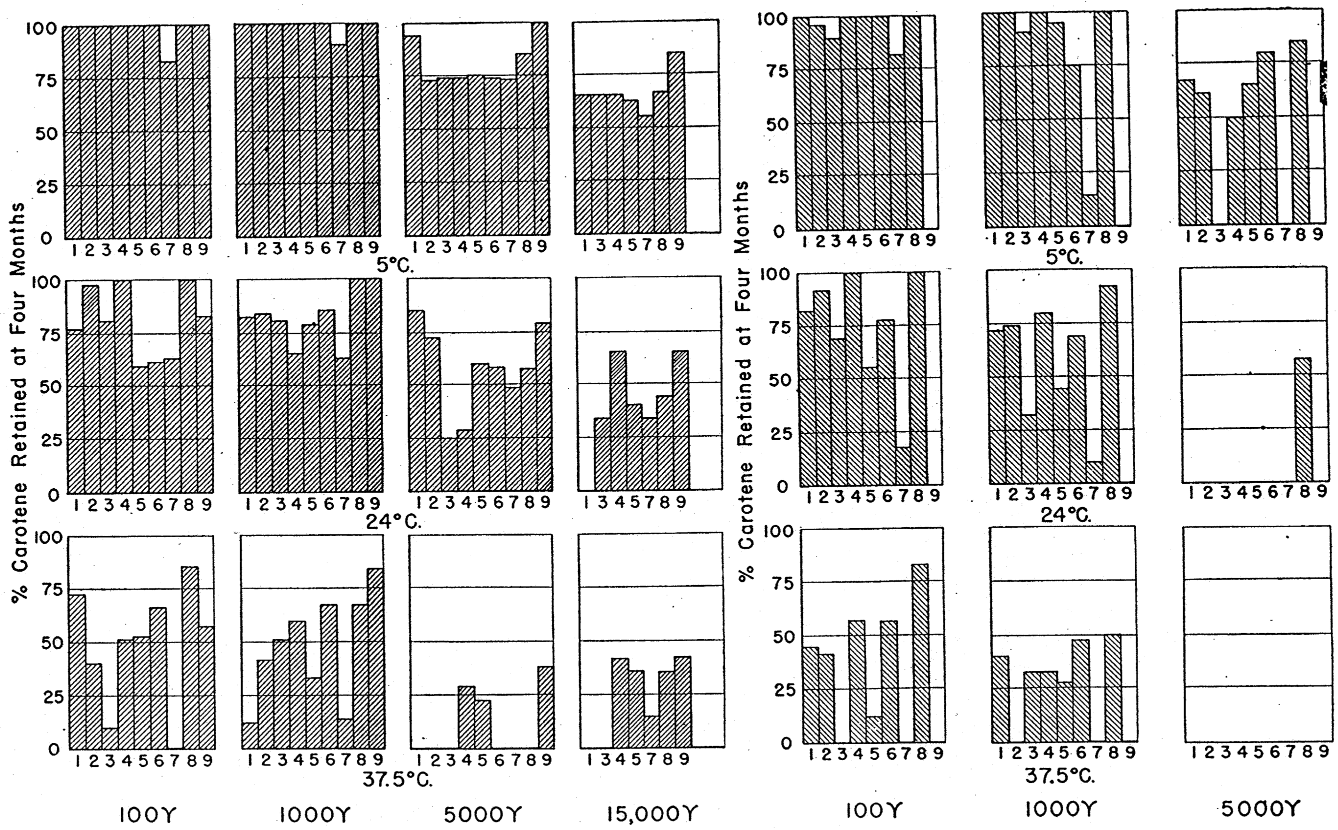

Figure 5. Carotene Retention in Oils after 4-Month Storage (Carotene Concentrations in Micrograms per Gram of Oil)

Four graphs at left, carotene concentrate; three graphs at right, crystalline carotene
1, refined cottonseed; 2, crude peanut; 3, refined peanut; 4, crude soybean; 5, refined soybean; 6, refined corn; 7, lard; 8, Crisco; 9, mineral oil

tory indicate that tocopherol was present in our carotene concentrate in relatively high concentration. Since tocopherols are effective antioxidants for carotene (*5, 11, 12, 20*), the greater stability of the leaf carotene concentrates may have been due to this factor.

The addition to the carotene in oil solutions of *d*-isoascorbyl palmitate and soybean lecithin, which increase the stability of lard and other fats (*12*), was ineffective with both crystalline and crude carotene at 5°, 24°, and 37.5° C. In contrast, studies by the Swift stability method at 100° C. indicated that these additives markedly increased carotene stability. It is apparent, therefore, that with this antioxidant combination, there was no correlation between the accelerated and normal carotene stability tests.

Our data indicate that, in many oils and fats in a concentration range of 100 to 1000 micrograms per gram, at least 50% of the original carotene will be retained for storage periods of 6 months at room temperature (24° C.). With higher carotene concentrations the oils must be refrigerated, and it is possible that greater stability could be obtained by storage under nitrogen or in evacuated containers.

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